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ASSESSING THE BIOLOGICAL THREAT POSED BY SUICIDE BOMBERS

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EXECUTIVE SUMMARY

The secondary threat of biological contamination with pathogenic organisms posed by suicide bombers is a significant knowledge gap. Studies of the 2005 London tube acts of terrorism highlighted the biological dimension to the threat, where the suicide bomber's bones acted as shrapnel and infected bystanders with hepatitis B. While it is unlikely the terrorist intended to spread this particular disease, we must now evaluate the possibility of intentional self-infection with highly contagious pathogens before a planned suicide bombing. Although infection by the implantation of bone fragments has been demonstrated in the past, the aerosolization of the remains of a bomber must be examined as well. Knowledge of the true contamination zone resulting from both aerosols and larger fragments from the bomber will aid first responders in preventing the risk of a potential epidemic by use of well directed decontamination and accurate determination of individuals exposed to potentially infectious agents.

To address the problem of secondary, infectious after effects of a suicide bombing, we performed a series of outdoor tests to determine if aerosols were generated that could harbor living organisms. *Our results demonstrate that aerosols generated can protect living organisms from the heat and pressure of an explosion and that those aerosols can be carried long distances, even upwind by the explosion.* To perform the tests, we used ballistic gelatin as test organisms were easily and uniformly distributed throughout each model used. Although more complex, and possibly more accurate, models of a suicide bomber were considered, budget and time constraints limited us to the ballistic gelatin blocks.

Three test scales were conducted, including small, medium, and large, respectively. The small test consisted of a PETN blasting cap with 2 g of high explosives. The medium scale tests were performed using a 1 lb. stick of military grade TNT. Finally, the large scale test utilized a mock suicide vest containing 8 one pound sticks of military grade TNT. Standard and high speed cameras captured the detonations, and samples were collected from the blasting pad for laboratory analysis. Further data was collected using APS and TAC-Bio devices. Aerosol particle counters demonstrated significant concentrations of particles originating from the ballistic gelatin, even upwind of the test. Furthermore, while sample plates showed significant bacterial contamination up to ~350 feet away from the test site, there was no visible contamination of gelatin. We concluded small aerosol particles had protected and carried organisms to the test plates, and that aerosols can magnify the biological contamination zone from a suicide bombing.

PREFACE

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ASSESSING THE BIOLOGICAL THREAT POSED BY SUICIDE BOMBERS

1. INTRODUCTION

1.1 Objective

Our major objective is to understand the biological threat posed by a suicide bomber. In addition to the kinetic energy damage from the bomb itself and shrapnel, it is possible for a suicide bomber to carry pathogenic organisms. The organisms can either be carried externally, or even within the bomber himself. The latter is especially troubling since many of the barriers that have traditionally made use of biological weapons difficult (e.g. keeping the infectious organisms alive until delivery), are no longer an impediment. To accomplish the major objective, we wish to determine what fraction of organisms survive the heat and pressure of the detonation of high explosive (HE) in a suicide bomb, especially in aerosol form, and the spatial extent of dispersal of living organisms. Both the survivability and the spread of organisms are essential to understanding the biological dimension of the suicide bomber threat.

1.2 Background

Studies performed in Israel and in London after the 2005 bombings highlighted the infectious biological dimension to suicide bombings due to implantation of bone fragments and exposure to the flesh and blood remains of the bomber. Israeli studies¹⁻² noted that bone fragments from some bombers tested positive for hepatitis B and it was believed that surviving victims had been infected by the bomber. Studies of the London 2005 bombings³⁻⁴ also observed extensive implantation of bone fragments. Due to the ability of the Hepatitis B virus to spread through wounds, testing and prophylaxis for hepatitis and other potential infections were recommended for people close to a suicide bombing. It is clear that bone fragments can harbor and protect infectious organisms from the heat and pressure of the initial HE blast. However, these studies have focused on the unintentional transmission of infectious disease. The threat of the intentional combination of infectious disease with a suicide bombing must be considered, as well as other potential mechanisms for infection, such as inhalation of aerosols generated from the bombing.

Other pathogenic organisms must be taken into account when considering the biological threat component of SBs. A somewhat recent Air Force study considered the threat of transmission of AIDS from a suicide bomber, showing the plausibility of the existence of a variety of biological threats.⁵ Organisms without available medical treatments or vaccines, such as Ebola, have also been considered as a potential bioterrorist material.⁶⁻⁸ There are technical barriers that make it difficult to manufacture the organisms in high concentrations as well as to stabilize and store the organisms in weapon. However, if an SB deliberately contracts the organisms, and travels to a destination while still asymptomatic, the two technical barriers above are easily surmounted. As a result, there could be catastrophic consequences in addition to the traditional aftermath of a SB. We contend that it is of the utmost importance to see what kind of fragments are produced in a SB, if some of these fragments are in the form of aerosols, if even

small particles can shield living organisms from the heat and pressure of the HE blast, and the extent of dispersion for the fragments.

Previous testing performed by the Pyrotechnics Branch with 26 grams of explosive and a 100 g gel block suggest fragments travel quite far. Our test is a continuation of that study scaled to larger gel blocks and larger HE charges. We contend that highly contagious diseases could be exploited in a suicide bombing, and that it is essential to understand the propagation of biological material from that event. To that end, we intend to understand the extent of the dispersion of body fragments, potential generation of aerosol that wind can transport, and whether any organisms within the bomber's remains stay viable, and obtain rough measurements of the fraction of biological material that surface the detonation of HE.

2. METHODS

2.1 Overview

There were a set of specific questions we sought to answer in our assessment of the biological threat from an SB. First, do organisms survive the thermal and blast effects of the HE detonation? Second, how does the survivability scale with the size of the HE blast? Third, for aerosol particles, how does particle size affect organism survivability? Lastly, how widespread could a contaminated area be? In order to answer these questions we endeavored to perform a test with a model SB that could be seeded with an organism, and then detonated under controlled conditions. Clearly, the human body is a complex mixture of different tissues, organs, bones, and fluids that each would have a unique response to the detonation event. Ideally, either a human or animal cadaver would provide the most realistic results.

We chose to utilize a very simple SB model composed of ballistic gelatin for a number of reasons. First, with ballistic gelatin, it is possible to uniformly seed the material with a test organism. The uniformity allows us to make a comparison between samples before and after the explosion and facilitates the interpretation of the data. Second, because a test of this kind has not been performed to the authors' knowledge, this was a range finding test to determine within an order of magnitude the kind of response to expect. Simplicity of the test was essential. Third, due to budget and time constraints, it was not practical to perform a full blown, complex test.

For the test organism, we utilized *Bacillus atrophaeus* (BG) spores for two reasons. First, we wanted to maintain a consistent concentration of organisms between different blocks of gelatin. We had little control of the time between preparation of the gel block and use in a test. We feared outright use of vegetative cells might significantly change the concentration of organisms during that lag time. Second, BG spores are readily available and covered by existing standing operating procedures (SOPs) at the test sites. We had considered using MS2 bacteriophages to simulate the behavior of viruses, yet modifying the SOPs to include MS2 would have prevented us from performing the program within the time allotted. As result, these initial range finding tests utilized only BG spores.

2.2

Gel Preparation

Based on guidance from the Army Research Laboratory's Survivability, Lethality, Analysis Directorate (ARL-SLAD), we utilized a 20% by weight formulation of natural porcine and bovine ballistic gelatin procured from Fisher Scientific. A food grade fluorescent dye (WaterGlo 801, Spectroline, Inc.) was added to act as a tracer. Distilled water was used to dissolve the gelatin. Unwashed “Danish milled” BG spores manufactured at the Dugway Proving Grounds was used as the test organism. Table 1 below shows the amounts of material used for each ballistic gelatin sample. For the small and medium scale tests, we did not possess a means of rigorously mixing the gelatin powder into the water. We found that mixing the powder into the water first followed by heating to 150o F ensured complete dissolution of the gelatin powder and a uniform liquid. For the large scale tests, involving ~25 kg of gelatin, a large oven set to 160o F was used to heat the water. Due to the time it took to heat ~25 L of water at once, the water was preheated and measured to ensure the correct concentration of gelatin. An industrial scale mixer was used to introduce the gelatin into the water and ensure the uniformity of the mixer. To avoid heat damage to the fluorescent dye tracer and the BG spores, these were not added to the liquid gelatin until the solution cooled to 120oF. The gelatin was poured into molds before being placed into a refrigerator. The solid ballistic gelatin melts around 105oF. For the large scale ballistic gelatin blocks, a 7 gallon waste basket lined with a plastic bag was used as a mold. This approximated the size of a torso of a small person.

Table 1: Ballistic Gelatin Formulation

| Test | Mass of Gelatin (kg) | Mass of Water (kg) | Volume of Dye (μ L) | Mass of BG for positive test (g) |
|------------|----------------------|--------------------|--------------------------|----------------------------------|
| Small (-) | 0.02 | 0.08 | 2 | 0.0 |
| Small (+) | 0.02 | 0.08 | 2 | 0.024 |
| Medium (-) | 0.43 | 1.72 | 40 | 0.0 |
| Medium (+) | 0.43 | 1.72 | 40 | 0.2 |
| Large (-) | 5.27 | 21.6 | 500 | 0.0 |
| Large (+) | 5.27 | 21.6 | 500 | 2.12 |

The purpose of the fluorescent dye was to serve as a quantitative tracer for collected small particles of the gelatin that were too small to measure with a mass balance. A fluorimeter with 365 nm excitation filter and 430 nm fluorescence emission filter was used to measure fluorescence from samples of gelatin and dye. It was found that the gelatin had intrinsic fluorescence, and so calibration curves were generated with a fixed amount of gelatin while varying the concentration of dye. The measurements were performed until the fluorescence emission saturated, and the concentration of dye in the gel was chosen to be before the plateau in the calibration curve. Given the known concentration of dye, it was intended to be able to scale fluorescence measurements to the mass of ballistic gelatin collected. Undiluted rinses from both the microscope slides and the swabs were measured with the fluorimeter. The amount of gelatin collected did not produce any measurable fluorescence signal. No additional fluorescence measurements were made.

To measure the amount of colony forming units in the ballistic gel before the explosion, for each test, a small sample of the gelatin was taken. The mass of the sample was measured, dissolved in water. The resulting solution was then plated onto agar plates, and the colonies counted. Three samples from different parts of the gel blocks were taken to demonstrate a uniform mixing of the BG into the gel.

2.3 Sample Collection

Samples were collected using three methods. The first method used color changing watch cards. These had to be fixed into place with double sided tape. Some vertical surfaces on reinforced concrete structures were available on the test pad, and some of the watch cards were fixed there. Watch cards were collected and bagged after each test. No further processing or handling occurred with the watch cards. The second method utilized plastic microscope slides measuring 1" x 2.5". Because the wind easily moved the slides, these were taped down to the same surfaces as the watch cards with double sided tape. At the end of each test, these were collected into 50 mL vials, and promptly placed in a cooler following the tests. To detect collection of viable BG, the slides were rinsed with a calibrated amount of ultrapure water, and the rinse was plated onto agar plates. An optical counter was used to count BG colonies. Lastly, glazed 8" x 4" tiles in conjunction with swabs were used to collect sample. These were sterilized with a bleach solution, rinsed with distilled water, and allowed to air dry. The swabs consisted of small wipes with a calibrated amount of ultrapure water. The rinse was plated onto Agar plates in the same manner as the microscope slides. Some visible pieces of gelatin were collected (prior to melting) directly into 50 ml vials and stored in an icebox.

Testing occurred over two days. The small scale, medium scale, and BG negative large scale shots were performed on the first day. The large scale test that included BG was done on the second day of testing.

Aerosol measurements were also performed using a standard TSI Aerodynamic Particle Sizer (APS) model 3321, and a TAC-Bio fluorescent particle counter. The standard APS relies on light scattering alone to detect particles, and thus detects all particles generated during the test regardless of composition. The TAC-Bio, on the other hand, utilizes both fluorescence and elastic scattering from a deep UV LED at 273 nm wavelength to detect aerosols. Because the ballistic gelatin is composed of protein and fluorescent dye, it is highly fluorescent so that the TAC-Bio only senses aerosols resulting from the ballistic gel. The APS was mounted on a table 1 m away from the ground. The TAC-Bio was mounted on a tripod approximately 1.5 m away from the ground. To avoid damage to the instruments, they were typically about 100 m away from test. These ran continuously during the tests while automatically logging aerosol count data.

2.4 Explosives testing

Three tests scales were performed, small, medium, and large. A PETN blasting cap with 2 g of HE was used for the small scale test. The gelatin was mounted on an inverted plastic cup. For the first shot, without BG, the blasting cap was placed next to the gel block. The result was very little fragmentation of the block, but a rather large piece got thrown ~10 m.

For the second test with BG, the blasting cap was inserted into the center of the block, resulting in much greater dispersion of the gelatin. For the medium scale tests, the gel block was placed on top of a cardboard can 1 m high and next to a 1 lb. stick of military grade TNT. For the large scale tests, the gel block was placed on top of a custom built wood platform (to minimize shrapnel for safety), so that it stood approximately man high. A model suicide vest was formed from 8 one pound sticks of TNT sewn to a vest made from a bath towel. The sticks were arranged four in front and four in back. The detonation cord was arranged so that the TNT would detonate in an even sequence. Both standard and high speed video cameras recorded the explosive events. The tests were conducted on the test range in the Aberdeen Proving Ground-Edgewood Area. The test was conducted on a concrete pad consisting of individual slabs measuring 30' x 12.5', forming a grid pattern of 12 by 24 slabs. This resulted in total pad dimensions of approximately 300' x 360'. The grid pattern facilitated mapping of the sample collection.

3. RESULTS AND DISCUSSION

3.1 High Speed Video



Figure 1: Still frames from first small scale test.

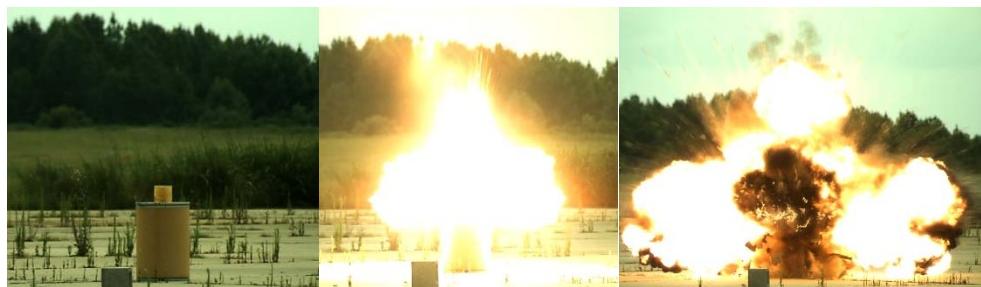


Figure 2: Still frames from medium scale test



Figure 3: Still frames from large scale test

The high speed video shows production of aerosol, as well as significant amounts of large scale chunks. Figures 1 to 3 show still frames of the different scale tests. For the small scale tests with the blasting cap, fragmentation of the gel block was poor, especially for the first test where the blasting cap was placed next to the gel. A large chunk approximately half the size of the original block was left over. For the medium scale test with 1 lb. of HE, the gel block was completely destroyed. In the last frame in Figure 2, significant aerosol and larger scale fragments are apparent in the video. As would be expected, much more aerosol is released with the large scale test. In the second still from in Figure 3, on the leading edge of the fireball, aerosol riding the edge of the explosion can be seen. In the last frame of Figure 3, the ejection of the larger scale particles are apparent around the remaining fireball.

3.2 Sample Collection Maps

Figures 4 to 6 show the locations of samples collected on the grid formed from individual slabs of concrete that formed the test pad area. Results from the small and medium scale runs are not shown because only the microscope slides could be collected from both gel blocks without BG (control) and blocks with BG (experiment). The microscope slides had to be anchored with double sided tape. We found that we could not easily remove the double sided tape, and as a result, dirt present on the pad also got rinsed and plated. The result was many non-BG bacterial colonies were detected, as well as significant contamination from previous tests. We found that the glazed tiles could be swabbed without any contact to the ground, thus greatly reducing the possibility of contamination from previous tests. As a result, only swab data, or directly sampled large pieces are reported.

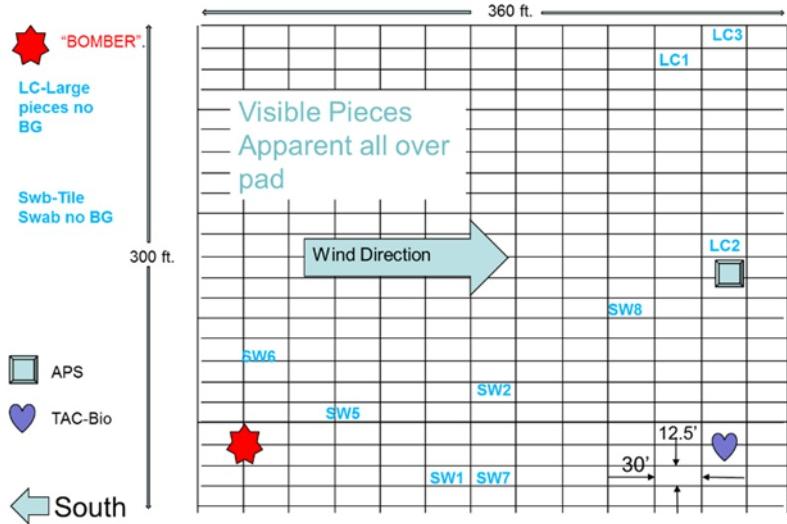


Figure 4: Sample collection map for large scale test without BG, showing the approximate locations of large sample collections ("LC" prefix), and swabbed tile locations (prefix "SW").

Figure 4 shows the results from the test control without BG. The locations of the APS and TAC-Bio sensors are shown on the map as well. The test explosion was positioned on the crossing of grid lines as shown in the map. The need to avoid damaging other structures on the pad dictated the location of the large scale test at the southeast corner. The direct sampled pieces of gel and glazed tiles were positioned on the grid as shown on the map, with visible pieces of gel covering the entire concrete pad. As should be expected, large pieces sampled showed no concentration of BG, most of the tiles showed zero BG colonies. Only three tiles showed BG colonies. One of the background tiles had been dropped on the ground, resulting in contamination, and was not considered for determining the background colony counts, with an average of 21 CFUs and standard deviation of 40 CFUs. The near absence of BG colonies indicate that positive detections of BG come from the ballistic gel alone. Had the BG come from the ground, from previous tests, or from contamination of the biolab, then addition of BG to the gel block should have led to a minimal difference in bacterial colonies.

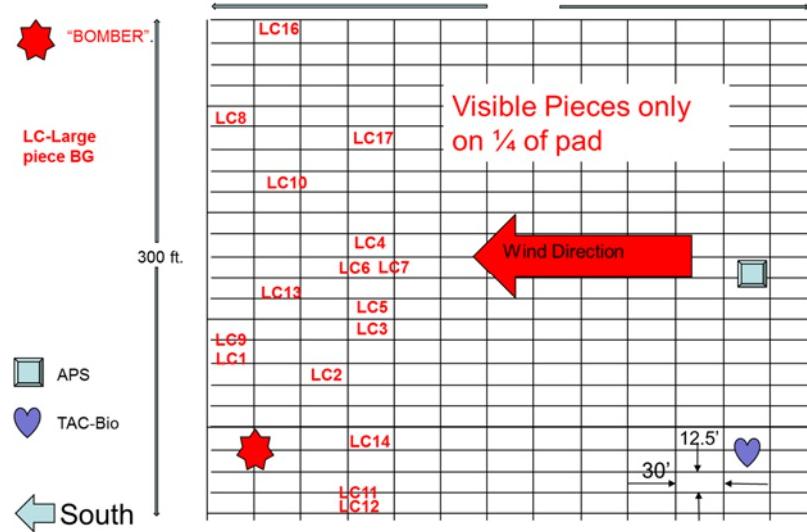


Figure 5: Sample collection map for large scale test with BG, showing the approximate locations of large sample collections ("LC" prefix) only. Due to the wind direction during the test, only the southern quarter of the pad had visible large pieces of gelatin.

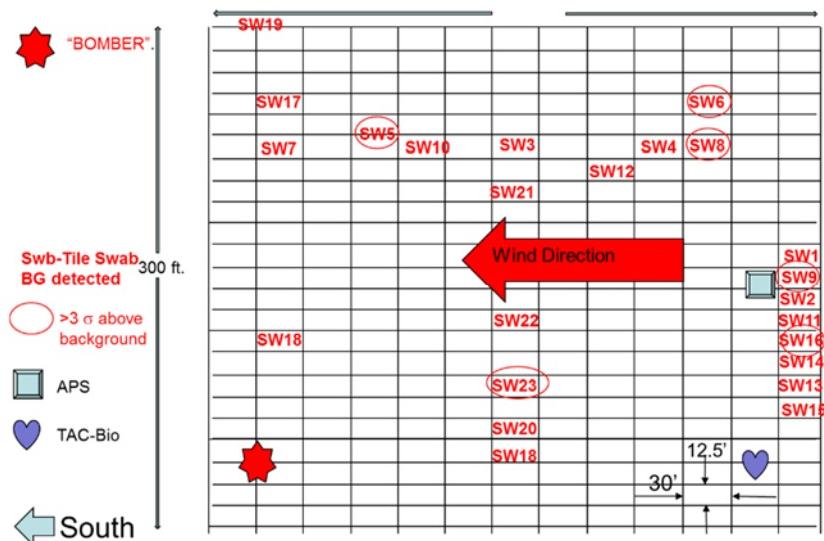


Figure 6: Sample collection map for large scale test with BG, showing the approximate locations of the swabbed tiles only. A number of tiles, even ones quite far from the explosion site, exhibited bacterial colonies of BG far above that observed for the background (tests with no BG in the gel). These locations are circled.

Tiles with the highest concentration of BG colonies were placed about 350' away from the blast site.

Figures 5 and 6 show the mapping of samples collected for the large scale test with BG introduced into the ballistic gel block. These are divided into two maps for the sake of the clarity. Figure 5 shows the direct sampling of visible pieces of gel. Although the outside of the pieces appeared to be singed, the visible bits of gel were solid and had not melted, despite the low melting temperature of $\sim 105^\circ \text{ F}$. Not surprisingly, these pieces protected the BG spores within quite effectively, so that on a mass to mass basis, there was negligible reduction in viable organisms. This is consistent with the Israeli and London bombing observations that bone fragments can transmit disease. Also, the wind direction had changed on the day we ran the BG positive large scale test, so that visible fragments were only visible on about a quarter of the pad.

No visible pieces or flecks could be seen on the sample collection tiles. These appeared to be uncontaminated.

Although we nominally expect large visible pieces of gel to shield organisms from the heat and pressure of the HE blast, our results show particles too small to see with the naked eye had placed viable bacteria on our sample collection points. Figure 6 shows the locations for the collection tiles that were swabbed. We attempted to place a line of tiles somewhat close to the test site to maximize the chance of collecting viable material, while at the same time leaving some of the tiles at the edge of the testing pad to capture how far material could travel. Because of the change in wind direction and because of the absence of visible material in the vicinity of the tiles, we expected no viable organisms to be collected at all. Surprisingly, more CFUs than the background average plus three times the standard deviation of BG colonies (3 sigma) were cultured from six tiles placed up to 350' away, upwind. As shown in Table 2, seven additional tiles also had BG colonies, but the numbers did not exceed the 3 sigma threshold. These results show that several sampling points were contaminated with organisms although there was no visible contamination.

Table 2 : Raw colony counts from large scale BG positive test

| Sample | CFU's/ ml |
|-----------|-------------|
| LSBG+Sw1 | 0 |
| LSBG+Sw2 | 0 |
| LSBG+Sw3 | 0 |
| LSBG+Sw4 | 66.6666667 |
| LSBG+Sw5 | 200 |
| LSBG+Sw6 | 1666.666667 |
| LSBG+Sw7 | 0 |
| LSBG+Sw8 | 533.3333333 |
| LSBG+Sw9 | 6533.333333 |
| LSBG+Sw10 | 0 |
| LSBG+Sw11 | 66.6666667 |
| LSBG+Sw12 | 0 |
| LSBG+Sw13 | 0 |
| LSBG+Sw14 | 133.3333333 |
| LSBG+Sw15 | 133.3333333 |
| LSBG+Sw16 | 266.6666667 |
| LSBG+Sw17 | 66.6666667 |
| LSBG+Sw18 | 66.6666667 |
| LSBG+Sw19 | 0 |
| LSBG+Sw20 | 0 |
| LSBG+Sw21 | 66.6666667 |
| LSBG+Sw22 | 0 |
| LSBG+Sw23 | 2666.666667 |

3.3 Aerosol Sampling

In order to make some measurement of the aerosol particles produced during the testing, both an APS and a TAC-Bio detector were employed. Because the APS detects any particle that elastically scatters light, upwards of hundreds of thousands of particles per liter are detected. Only fluorescent gelatin particles appear in the TAC-Bio readout, and so fewer particles are detected. Neither the APS nor the TAC-Bio had been configured to detect BG spores embedded in the gelatin particles.

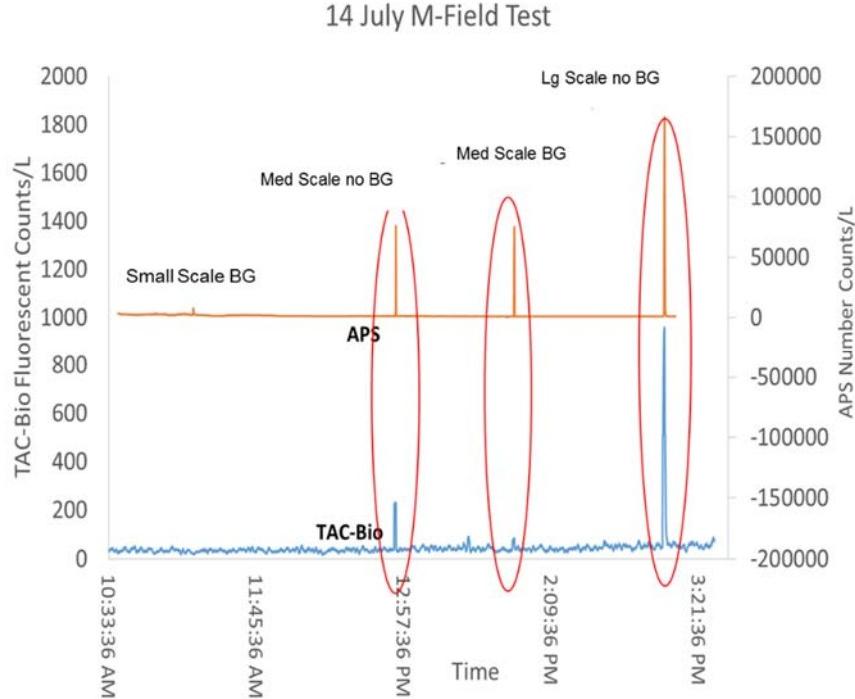


Figure 7: APS and TAC-Bio data for the first day of testing. The large scale BG negative test appears as the last event in the chart. TAC-Bio data scale appears on the left, while the APS scale appears on the right axis. TAC-Bio only senses the gelatin particles.

Figure 7 shows the output of both the APS and the TAC-Bio for the first day of testing. Because both sensors were not collocated, they do not necessarily observe the same events. None of the small scale tests produced a response in the TAC-Bio, although the sensor was not downwind. Only one of the small scale tests appear in the APS data. Both medium scale shots appeared in the data, although weakly in the TAC-Bio data. These events appear as sharp spikes in the particle counts. This is not surprising given the breezy conditions, so that particles kicked up by the explosion were removed from the test site within a minute or so. For the APS, which detects all particles generated, upwards of 150,000 particles per L were detected for the large scale test. At the same time, approximately 1000 particles per L were detected with TAC-Bio for the same test. These sensors were downwind of the test, and so many particles were detected.

Aerosol measurements performed on the day of the large scale BG positive test show that it is most likely the viable bacteria collected on the tiles were most likely carried by

aerosol particles. Figure 8 shows the results of the second day of testing when the wind direction changed and both sensors were then upwind of the test. As might be expected from the dispersion of visible gelatin chunks away from the sensors, the APS shows no increase in particulates. However, because the TAC-Bio is selective to the gelatin particulates, it is able to see a 200 particle per liter increase due to the test. We attribute the attenuation in particle counts on the TAC-Bio and APS to the wind direction. Although the collection tiles and the TAC-Bio sensor were upwind of the test by about 350', it is clear some aerosol particles “surfed” the shockwave from the blast and were carried a distance upwind. Had the sampling surfaces been downwind, we expect the amount of bacterial contamination would have been more extensive.

We believe these results answer at least two of the questions posed at the beginning. The detection of BG colonies on sampling tiles far upwind of the test site show that organisms can be shielded and protected by aerosol particles. Given that surfaces could be contaminated without visible chunks, it can be seen there is a significant potential threat. Aerosol measurements show that particles that can only come from the ballistic gelatin are generated, and we must attribute the delivery of viable organisms to the sample surfaces to these particles.

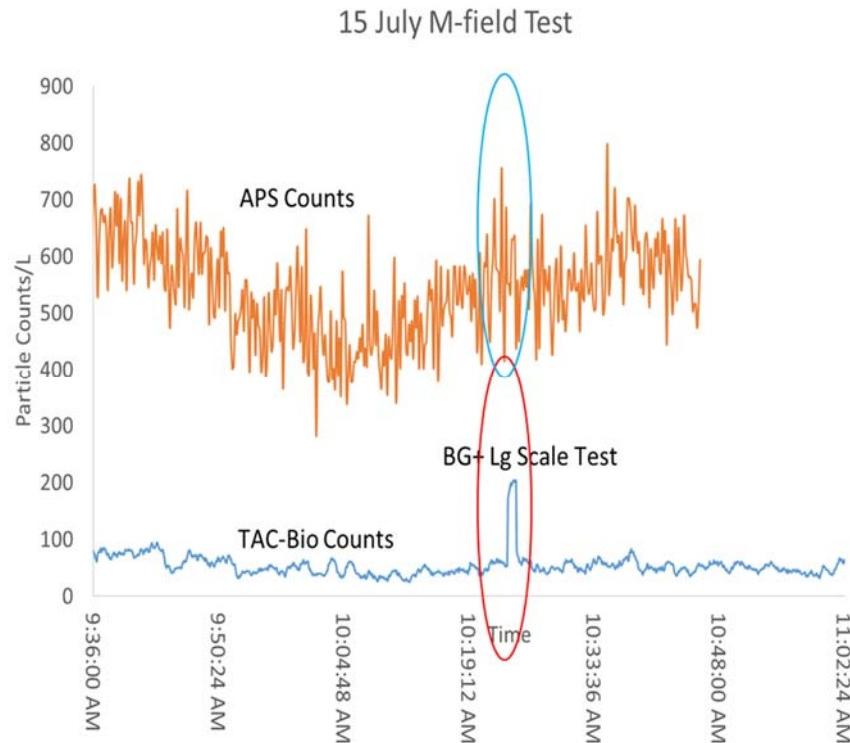


Figure 8: APS and TAC-Bio data for the second day of testing with the large scale, BG positive test. Because of the wind direction, no additional particles relating to the test were detected, and so both outputs appear on the same scale.

Although our results show that some aerosols are generated from the test SB, and that these particles can harbor viable organisms, we believe the threat could be much worse than indicated. First, the ballistic gelatin only approximates the mechanical properties of muscle tissue. The activity of blood and other precious bodily fluids must be considered, because they can aerosolize much more easily. Our expectation is that more aerosol would result from a more realistic test.

4. CONCLUSIONS

Within the limitations of using ballistic gelatin as a model of a suicide bomber, we demonstrated that even small, aerosol sized pieces of gel can protect organisms from the effect of the high explosives blast. We show that these small particles containing organisms can be hurled hundreds of feet by the blast. We expect aerosolization from a real human who contains blood and other bodily fluids that will aerosolize even more material. We intend to seek additional funding to continue finer grained tests that incorporate other organisms, such as MS2 virus, or vegetative E. coli, to assess how survivability changes based on the threat organism.

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ACRONYMS AND ABBREVIATIONS

| | |
|----------|-----------------------------------------------------------------------------|
| AIDS | Autoimmune Deficiency Syndrome |
| APS | Aerodynamic Particle Sizer |
| ARL-SLAD | Army Research Laboratory-Survivability, Lethality, and Analysis Directorate |
| BG | Bacillus globigii (Bacillus atrophaeus) |
| CFU | Colony Forming Unit |
| HE | High Explosive |
| LC | Large Chunk, large piece of ballistic gelatin collected |
| LED | Light Emitting Diode |
| TNT | 2,4,6-trinitrotoluene |
| PETN | Pentaerythritol tetranitrate |
| SB | Suicide Bomber |
| SOP | Standing Operating Procedure |
| SW | Swab |
| UV | Ultra Violet |

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